Journal of Chromatography, 307 (1984) 399—403
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2030

Note

Determination of creatinine in human, dog and rat urine by high-performance liquid chromatography on a column of hydroxymethylated porous polystyrene

AKIRA YOSHIDA and YUKO YAMAGUCHI

Central Research Laboratory, Mitsubishi Petrochemical Co., Ami, Inashiki, Ibaraki 300-03 (Japan)

and

MASANORI YOSHIOKA*

Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotogecho, Hirakata, Osaka 573-01 (Japan)

(First received June 8th, 1983; revised manuscript received December 8th, 1983)

Creatinine in urine is commonly determined by assay methods based on the Jaffé reaction [1] in which creatinine reacts with alkaline picrate to form an amber-yellow colour. This reaction, however, is non-specific and many compounds are known to react with picrate [2]. Preliminary purification by ion-exchange chromatography has been attempted to overcome these difficulties [3—5]. More specific enzymatic methods have also been used [6].

Recently, high-performance liquid chromatography (HPLC) using a reversed-phase column was introduced for the determination of creatinine in human urine and plasma [7—13]. These HPLC separations are fast, reliable and require minimal work-up. However, a method for the determination of creatinine in urine of experimental animals such as dog and rat has not been reported.

In this paper we describe a method for measuring creatinine in human, dog and rat urine by HPLC on a column of hydroxymethylated porous polystyrene which is chemically more stable than the regular reversed-phase column.

EXPERIMENTAL

Materials

The column packing material was Hitachi Gel No. 3013-O (Hitachi, Tokyo,

0378-4347/84/$03.00

Japan) made of spherical porous particles of a hydroxymethylated styrene—divinylbenzene copolymer with an average particle diameter of 3 μm.

Creatinine was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Creatinine test kit based on the Jaffé reaction was from Wako Pure Chemical Industries (Osaka, Japan).

Apparatus

A high-performance liquid chromatograph Jasco Trirotar—II (Japan Spectroscopic Co., Tokyo, Japan) equipped with a variable-wavelength ultraviolet detector Uvidec-100-II (Japan Spectroscopic Co.) was used. Absorbance was continuously recorded at 236 nm.

The packing material, dispersed in distilled water, was packed into stainless-steel tubing at a pressure of about 150 kg/cm^2 by the slurry technique.

Sample urine

Human (male, age 29—38 years, weight 58—86 kg) urine was collected in a polystyrene bottle during 24 h. Rat Wistar ST (male, age 9 weeks, weight 210—262 g) urine was collected in a metabolic cage over a 24-h period. Beagle dog (male, age 8 weeks, weight 10—12 kg) urine was collected in a glass tube using a cannula. These urine specimens were stored at −80°C until analysis.

Procedure

The urine was diluted 25-fold with distilled water. Aliquots (5 μl) of the resulting solution were injected into the liquid chromatograph and analysed under the conditions described in the legend to Fig. 1. The urinary level of creatinine was quantitated from its peak height on the chromatogram.

The colorimetric determination of urinary creatinine based on the Jaffé reaction was performed using the creatinine test kit.

RESULTS AND DISCUSSION

Separation

Figs. 1, 2 and 3 show the typical results obtained for the analysis of human, dog and rat urine, respectively. Uric acid, trigonelline and pseudouridine were eluted as shown in Fig. 1. Hippuric acid largely excreted in urine was eluted at 26.4 min. Furthermore, creatine phosphate and also creatine were separated from creatinine. Thus, these constituents did not interfere with the determination of creatinine in this present HPLC method.

The separation could be carried out with better selectivity and resolution by using hydroxymethylated porous polystyrene of 3- or 5-μm particle diameter such as No. 3013-O than by using particles of 10—12 μm diameter such as No. 3011-O. No. 3013-O (3-μm particle diameter), which showed a slightly higher resolution than that of the 5-μm diameter particle, was adopted in our studies. The symmetry of the creatinine peak on this column was better than that obtained by employing the conventional C_{18} reversed-phase column. Retention time did not change over a period of ten months.

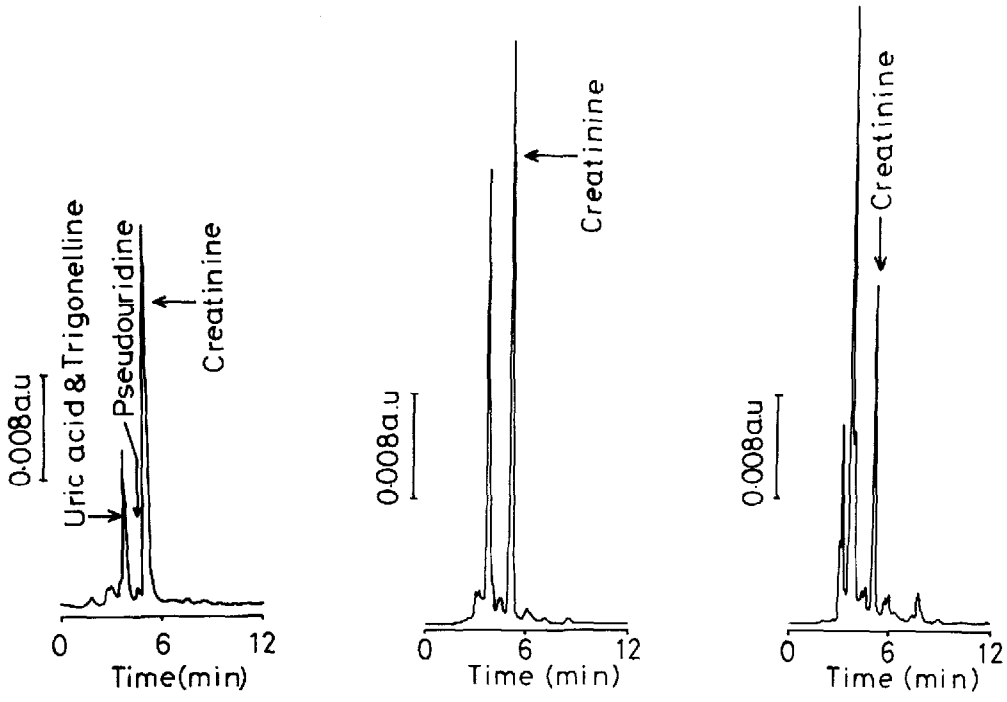

Fig. 1. Chromatogram of urine from a normal person. Urine was diluted 25-fold with distilled water. A 5-μl volume of this solution was applied under the following conditions. Column: stainless-steel tubing (250 × 4.6 mm I.D.) packed with Hitachi Gel No. 3013-O (particle diameter about 3 μm) at 30°C. Eluent: 0.02 *M* ammonium carbonate aqueous solution. Flow-rate: 0.8 ml/min. Detector: 236 nm, 0.08 a.u.f.s.

Fig. 2. Chromatogram of urine from a beagle dog. For conditions see legend to Fig. 1.

Fig. 3. Chromatogram of urine from a Wistar ST rat. For conditions see legend to Fig. 1.

Working curve, precision, recovery and accuracy

The relationship between peak height and amount injected into the chromatograph was linear over the range of 25—500 ng. The sensitivity of the assay was 38 mg/l with a signal-to-noise of 10.

The reproducibility of the present method summarized in Table I was fairly good compared with that of HPLC using the reversed-phase column [8, 13].

Portions of a urine containing 0.63 g/l creatinine were augmented to achieve final concentrations of 1.13 and 1.63 g/l. Analytical recovery (mean ± 1 S.D.) was 99.7 ± 0.5% (n = 5) and 100.3 ± 0.4% (n = 5), respectively.

Ultraviolet spectra of fractions corresponding to the chromatographic peaks of creatinine from column were identified for both urinary and reference creatinine as shown in Fig. 4. The correlation between the present method and the colorimetric method based on the Jaffé reaction was high (Fig. 5). For the urinary excretion of creatinine in dog and rat, however, the colorimetry gave results that were somewhat high in comparison with those obtained by HPLC. These results strongly support the present method as reliable for practical purposes and especially useful for the determination of creatinine in urine of various experimental animals.

TABLE I

PRECISION OF CREATININE ASSAY

	Conc. (g/l)	n	C.V. (%)
Within-day	0.63	6	0.7
	1.90	6	0.4
Between-day	0.63	10	2.7
	1.90	10	1.6

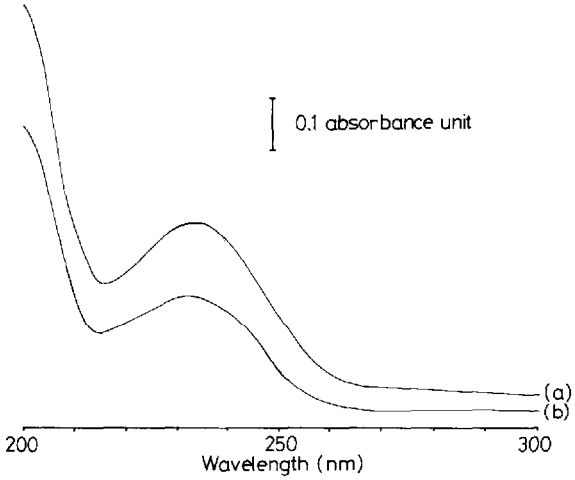

Fig. 4. Ultraviolet spectral comparison of urinary (a) and reference (b) creatinine. The same urine as in Fig. 1 was used. Fractions corresponding to chromatographic peaks of creatinine from the column were combined and freeze-dried to remove the ammonium carbonate. The samples were then dissolved in 4 ml of distilled water and their ultraviolet spectra measured. The spectrum was scanned from 300 to 200 nm at 120 nm/min scan speed and 2.0 nm slit width with a Hitachi 557 spectrophotometer (Hitachi, Japan).

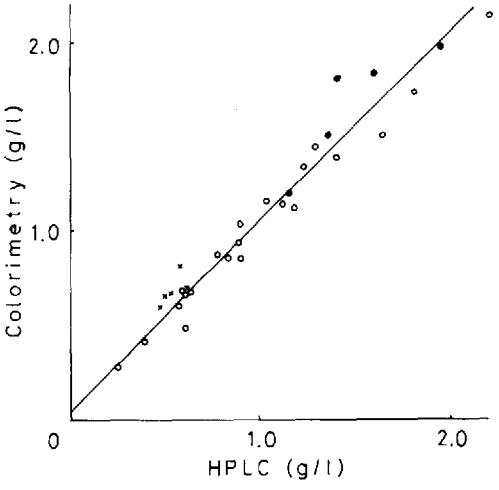

Fig. 5. Comparison of the present procedure with the method based on the Jaffé reaction for urinary creatinine. n = 31, r = 0.975, slope = 1.013, intercept = 0.032. (○), Human; (×), Wistar ST rat; (●), beagle dog.

Human urine

The urinary excretion (mean ± 1 S.D.) of creatinine in six normal humans was 1580 ± 150 mg/day (1410—1830 mg/day). The excretion (mean ± 1 S.D.) per g body weight was 23.3 ± 2.3 μg/day, which was consistent with values reported in the literature [14].

Dog urine

The concentration (mean ± 1 S.D.) of creatinine in urine of five beagles was 1490 ± 290 mg/l (1160—1950 mg/l).

Rat urine

The urinary excretion (mean ± 1 S.D.) of creatinine in five Wistar ST rats was 6.69 ± 1.60 mg/day (5.38—9.09 mg/day). The excretion (mean ± 1 S.D.) per g body weight was 28.0 ± 4.5 μg/day, which was larger than that excreted by humans.

CONCLUSION

The present method is very useful for the determination of urinary levels of creatinine not only in humans but also in dog and rat. The column used has a long life and is therefore economical for routine use.

ACKNOWLEDGEMENTS

We are deeply grateful to Dr. S. Mizogami and Mr. T. Higuchi of Mitsubishi Yuka Pharmaceutical Co. Ltd. for supplying dog and rat urine samples and to Dr. M. Uchiyama and Mr. T. Sakai of Mitsubishi Petrochemical Co. Ltd. for giving their support to this work.

REFERENCES

1 M. Jaffé, Hoppe-Seyler's Z. Physiol. Chem., 10 (1886) 391.
2 J.G.H. Cook, Ann. Clin. Biochem., 12 (1975) 219.
3 A.C. Teger-Nilsson, Scand. J. Clin. Lab. Invest., 13 (1961) 326.
4 J.A. Owen, B. Iggo, F.J. Scandrett and C.P. Stewart, Biochem. J., 58 (1954) 426.
5 A. Chason, H.J. Grady and M.A. Stanley, Amer. J. Clin. Pathol., 35 (1961) 83.
6 G. Szasz and U. Bőrner, Clin. Chem., 23 (1977) 1172.
7 C.K. Lim, W. Richmond, D.P. Robinson and S.S. Brown, J. Chromatogr., 145 (1978) 41.
8 S.J. Soldin and J.G. Hill, Clin. Chem., 24 (1978) 747.
9 W.L. Chiou, M.A.F. Gadalla and G.W. Peng, J. Pharm. Sci., 67 (1978) 182.
10 D.N. Buchanan, S.K. Tait and E.F. Domino, J. Chromatogr., 163 (1979) 212.
11 A.M. Krstulovic, L. Bertani-Dziedzic and J.M. Capurusso, Clin. Chim. Acta, 99 (1979) 189.
12 R. Achari, M. Mayersohn and K.A. Conrad, J. Chromatogr. Sci., 21 (1983) 278.
13 F.W. Spierto, M.L. MacNeil, P. Culbreth, I. Duncan and C.A. Burtis, Clin. Chem., 26 (1980) 286.
14 D.J. Greenblatt, B.J. Ransil, J.S. Harmatz, T.W. Smith, D.W. Duhme and J. Koch-Weser, J. Clin. Pharmacol., 16 (1976) 321.